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Environment supersedes host lineage in shaping the gut microbiome of tree squirrels in the Pacific Northwest

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BACKGROUND

- Vancouver Island ("VI") *Tamiasciurus hudsonicus* (red squirrels, "TH") are genetically more closely related to interior mainland TH in the Pacific Northwest, despite their closer geographic proximity to coastal *T. douglasii* (Douglas squirrels, "TD")[1].
- Studies show that both host genetics and environment influence the establishment and composition of the gut microbiome.
- However, it is unclear whether host genetics or the environment has a stronger effect in shaping the gut microbiome.

Figure 1. Distribution of *Tamiasciurus* collection sites in VI (left) through interior mainland Washington (right). Red points correspond with TH, and blue points, with TD. Oregon TD collection sites are not shown.

OBJECTIVES

To determine whether host lineage or environment drives gut microbiome composition of squirrels in the Pacific Northwest, we:

- compared mainland versus island microbiome compositions.
- compared congeneric squirrel microbiome compositions.

PREDICTIONS

1) If the environment has a stronger effect, then the gut microbiomes of VI TH will resemble those of coastal TD.
2) If host lineage has a stronger effect, then the gut microbiomes of VI TH will resemble those of interior mainland TH.

METHODS

- 35 TH (13 VI and 22 mainland) and 34 TD specimens collected from various sites in Vancouver Island, Washington, and Oregon between 2008-2010 (Fig. 1)
- DNA extracted from cecal samples with QIAamp DNA Stool Mini Kit[2]
- Amplified V4 region of 16s rRNA geneAmplicon[3]
- Utilized World Wildlife Fund's terrestrial ecoregions of the world classifications for grouping by environment conditions[4]

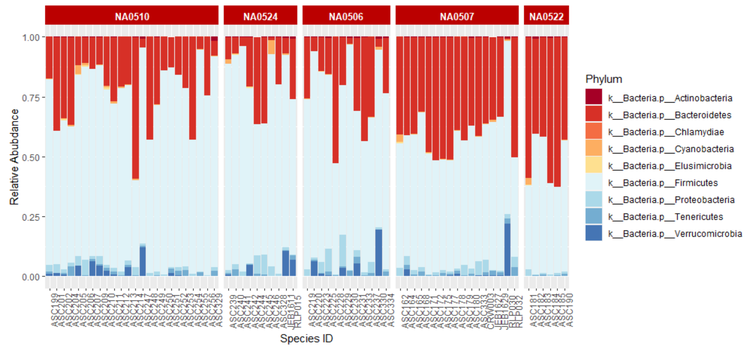

Figure 2. Taxonomic bar plot at the phylum level. From left to right: ecoregion NA0510 = VI TH and coastal TD (Oregon), NA0524 = coastal TD (Washington and British Columbia), NA0506 = coastal TD (Washington), NA0507 = interior mainland TH (Washington), NA0522 = interior mainland TH (Washington).

RESULTS

- Samples showed no variation in alpha-diversity metrics when considering sex, sampling season, and species assignment.
- Interior mainland TH have a greater relative abundance and consistency of *Bacteroidetes* compared to TD and VI TH (Fig. 2).
- Bray-Curtis dissimilarity and weighted Uni-Frac distance showed clustering of VI TH with coastal TD rather than with interior mainland TH (Fig. 3).
- Permutational analyses of variance showed greater dissimilarity between VI TH and interior mainland TH (q-value = 0.0025).
- Homogeneity of multivariate dispersion tests showed no significant difference between all groups (q-value > 0.05).

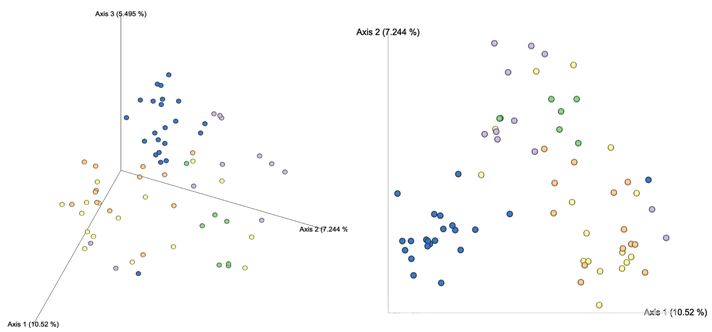

Figure 3. Principal coordinate analysis plots of weighted Uni-Frac distance (left) and Bray-Curtis dissimilarity (right). Blue = interior mainland TH, orange = VI TH, yellow = coastal TD (Washington), purple = coastal TD (Oregon), green = coastal TD (British Columbia). Axis 1 explains most of the variance in both plots (weighted Uni-Frac = 9.68%; Bray-Curtis = 10.52%).

CONCLUSIONS

- Gut microbiome compositions of VI TH squirrels more closely resemble those of coastal TD squirrels.
  → supports prediction 1 (Fig. 4)
- Significant difference in variance between VI TH and interior mainland TH is due to differences in microbial community and not an artifact of heterogeneous dispersion.

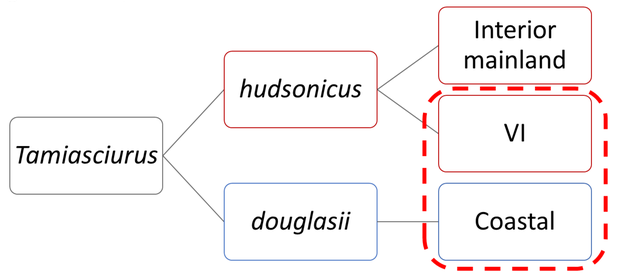

Figure 4. Prediction 1: environment has stronger effect on gut microbiome. Despite genetic relatedness to interior mainland TH, VI TH will have gut microbiome compositions that resemble those of coastal TD (predicted relationship circled).

DISCUSSION

- Both host genetics and environment shape the gut microbiome, but it is unclear which of the two has a stronger effect.
- Our findings from this natural reciprocal transplant system support a growing number of studies that identified environment as the stronger driver of gut microbiome composition.
- Additional investigation is required to identify any bacterial genera that are indicative or predictive of host species or environment.
- Additional investigation is required to determine the interaction between major gut microbiota phyla and genera on the host and vice versa.

LITERATURE CITED

1. Chavez et al. 2014. *Evolution, 68*(4):1094-1109; 2. Smith et al. 2011. *Open Microbiology Journal, 5*:14-17; 3. Caporaso et al. 2010. *Nature Methods, 7*:335-336; 4. Olson et al. 2012. *Bioscience, 51*(11):933-938.

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